

THE EFFECTS OF PHYSICAL VARIABLES AND ACCLIMATION ON SURVIVAL AND OXYGEN CONSUMPTION IN THE HIGH LITTORAL SALT-MARSH SNAIL, *MELAMPUS BIDENTATUS* SAY.¹

ROBERT F. MCMAHON² AND W. D. RUSSELL-HUNTER³

²Department of Biology, Box 19498, The University of Texas at Arlington, Arlington, Texas 76019;

³Department of Biology, Syracuse University, Syracuse, New York 13210; and ^{2,3}The Marine Biological Laboratory, Woods Hole, Massachusetts 02543.

ABSTRACT

The physiological ecology of the salt-marsh pulmonate gastropod, *Melampus bidentatus* Say, was investigated in specimens from the Little Sippewisset salt-marsh, Cape Cod, Massachusetts. *M. bidentatus* is tolerant of submergence, surviving 2-3 days at 20°C and 14 days at 10°C in 25%-100% seawater (SW). Snails acclimated at 10°C tolerated temperatures of 32.2°C-37.4°C, and snails acclimated at 20°C tolerated 35.2°C-40.7°C, when submerged in 0%-100% SW. LD (T)₅₀ in air was 44.5°C (10°C acclimated) and 44.7°C (20°C acclimated). Aerial weight-specific O₂ consumption ($\dot{V}O_2$) was 3-5 times higher than aquatic rates. Aerial and aquatic $\dot{V}O_2$ was regulated ($Q_{10} < 1.5$) over the ambient temperature range in both acclimation groups. The $\dot{V}O_2$ of 10°C acclimated individuals was lower than 20°C acclimated specimens, a pattern of "reverse" acclimation associated with energy stores conservation. O₂ debt occurred after 5 h anoxia. In addition, *M. bidentatus* compensates $\dot{V}O_2$ for hypoxia by increasing both uptake rates and the degree of O₂ regulation of $\dot{V}O_2$ during decreasing O₂ concentrations. *M. bidentatus* displays a circadian rhythm associated with desiccation pressures, and thus $\dot{V}O_2$ is maximal during evening and minimal during daylight hours. Under the selection pressures associated with longterm aerial exposure in the high littoral salt-marsh environment, *M. bidentatus* has evolved the vast majority of physiological adaptations required for terrestrial life, except for its aquatic egg-masses and planktonic development.

INTRODUCTION

Pulmonate snails of the genus *Melampus* live in the highest levels of the marine intertidal and are quite literally amphibious. *Melampus bidentatus* Say is found, often abundantly, in the higher levels of salt-marshes along the Atlantic coast of North America. In its reproductive activities, *M. bidentatus* is a primitive marine snail, spawning large numbers of small eggs which yield planktonic veliger larvae on hatching. Thus, all aspects of reproduction and early life-history have to be temporally "fitted" to the 2-week periodicity of spring tides (Russell-Hunter *et al.*, 1972). In contrast, in its respiration, this species is a land snail breathing air though

Received 28 May 1981; accepted 29 July 1981.

Abbreviations: HCT, heat coma temperature; L:D, light:dark (ratio); LD(T)₅₀, lethal dose (level) of temperature for 50% of experimental sample; MBL, Marine Biological Laboratory; SW, seawater; $\dot{V}O_2$, weight-specific O₂ consumption rate.

¹Supported by Grants GB-36757 and DEB-78-10190 from the National Science Foundation to W. D. Russell-Hunter; and by Research Grant 15-653 from Organized Research Funds of the University of Texas to R. F. McMahon.

pneumostome and lung, with no vestige of a ctenidium (gill) in its pallial organization. In this part of its physiology, *Melampus* can best be regarded as an air-breathing snail colonizing the high littoral environment and subjected to variable (but usually short) periods of submergence, variable salinities, and generally high and variable environmental temperatures. This provides a somewhat different adaptational background for the respiratory schesis of *Melampus* than that of the various species of littorinids, gill-bearing marine prosobranch snails, which show colonization of successively higher levels in the intertidal (McMahon and Russell-Hunter, 1977). The investigations reported here on the physiology of *Melampus* assess capacity and resistance responses to temperature and salinity stresses (corresponding to those experienced in the high littoral salt-marsh), and measure survivorship and rates of oxygen uptake at a variety of temperatures and salinities. Both respiratory and survivorship studies involved comparisons between stocks acclimated at different temperatures, and both aquatic and aerial respiration rates were measured.

MATERIALS AND METHODS

Field collections

Specimens of *Melampus bidentatus* were collected from the upper levels (*Spartina-Juncus-Distichlis* zone) of Little Sippewisset salt marsh on Buzzards Bay, just north of Woods Hole, Cape Cod, Massachusetts. Details of the physical and biotic environment of this natural *Melampus* population are provided in Apley (1970) and Russell-Hunter *et al.* (1972). Two collections each of more than 200 individuals were made, on 5 June and 9 June 1975, both at 1430 h. Ambient air temperature was approximately 17°C, surface water temperature in marsh pools 25°C, and subsurface (6 cm) water temperature 23°C, during these two collecting periods. All specimens of *Melampus* were taken at low tide from the muddy marsh surface about the bases of the spike-grass, *Distichlis spicata*. Egg masses of *Melampus* were abundant indicating the population was in a semilunar cycle of egg-laying (see Apley, 1970, and Russell-Hunter *et al.*, 1972, for a detailed discussion of life-cycle and reproduction in *Melampus*). Specimens were carried to the laboratory in insulated jars immediately after collection and placed in 20.3-cm-diameter glass culture bowls (approximately 100/bowl) previously lined with two sheets of 18.5 cm diameter filter paper soaked in full strength seawater. The wet lining was replaced every 3 days throughout the experimental period. The specimens of *Melampus* were divided into two groups, maintained in incubators at 10°C ($\pm 0.5^\circ\text{C}$) and at 20°C ($\pm 0.5^\circ\text{C}$) until utilized in experiments. Both groups were fed an excess of dried lettuce. Neither showed any significant mortality after maintenance under these conditions for more than 50 days.

Submergence tolerance

Melampus is an amphibious pulmonate, primarily air-breathing, being submerged only at high water of spring tides. To determine submergence tolerance in this species, one portion of the sample collected on 9 June 1975 was maintained in air for 2 days after collection at room temperature (21–25°C). Subsequently, subsamples of 10 specimens each were placed in 30 ml of MBL (Marine Biological Laboratory) artificial seawater at five salinities: 100% SW (salinity = 31‰), or 75% (=23.25‰), or 50% (=15.5‰), or 25% (=7.75‰) (see Cavanaugh, 1956, for

a detailed account of the chemical composition of MBL artificial seawater), or in Falmouth tap-water previously aerated for 3 days ($= <0.2\%$).

Nylon screens of 0.1 mm mesh size were secured with plastic snap rings in 40 ml centrifuge tubes (outside diameter = 3.85 cm, height = 11.8 cm) just below the water surface (30 ml volume), preventing aerial emergence by the snails but allowing free gas exchange across the air-water interface. Groups of snails were maintained submerged in this manner at either 10° or 20°C ($\pm 0.5^\circ\text{C}$) for 1, 2, 3, 4, 6, 8, 10, or 14 days. After each submergence period, all water was emptied from the tubes and the snails allowed a 24 h aerial recovery period. The number of snails attached to the walls of each tube then were recorded as alive, and those unable to reattach were recorded as dead.

Effects of salinity on thermal tolerance

The thermal tolerance of submerged snails was determined as mean "heat coma temperature" (HCT) after the method of McMahon (1976) used for a similar sized freshwater pulmonate snail, *Physa virgata*. Heat coma is a reversible condition characterized in molluscs by loss of nervous integration. It is manifested in *Melampus* by cessation of locomotory activity, a ventral-lateral curling of the foot, and inability to remain attached to the walls of the glass tube, a response similar to that of *Physa virgata* (McMahon, 1976; McMahon and Payne, 1980). The basic experiment consisted of placing five specimens in each of four to six 40-ml centrifuge tubes (as described for submergence experiments) with 30 ml of the aquatic medium under test (100%, 75%, 50%, or 25% MBL artificial seawater, or tap-water). The tubes were sealed with porous foam plugs that prevented snails from emerging. Temperatures in the tubes were monitored with a YSI Model 43-Td Tele-Thermometer. Water temperatures in the tubes initially equaled that of acclimation (10°C or 20°C). They were then raised in a block heater 1 degree every 5 min (± 0.5 min), a rate of increase that keeps the body temperature of marine littorinid snails far more massive than *Melampus* close to that of the medium without time lag (Broekhuysen, 1940; Evans, 1948). At each 1-degree increase in temperature the number of specimens entering "heat coma" was recorded. Death due to thermal stress usually occurred 3–4°C above HCT in *Melampus*.

In a preliminary experiment, snails from the field were maintained in air at room temperature (21–25°C) as described for 2 days. The shell lengths of 25 individuals were measured to the nearest 0.1 mm with a dial caliper. These measured snails were placed in six 40-ml centrifuge tubes (five tubes of four snails and one tube of five) in size groupings that allowed individuals to be identified visually by shell length. The remaining sample (of about 400 snails) was divided evenly, and the groups maintained at 10°C or 20°C. Subsamples of 20 individuals from each group were tested in full strength MBL seawater after 4, 8, 12, and 16 days and mean HCTs determined. After 19–20 days, mean HCT was also determined for subsamples of 20 individuals from each group in 75%, 50%, 25% MBL artificial seawater and tap-water. It proved impossible to determine the HCT of specimens of *Melampus* in air, as all snails withdrew into the shell at higher temperatures. Therefore, the upper lethal limit of snails in air was determined as the LD(T)₅₀, or the lethal dose (level) of temperature for 50% of the sample, for groups acclimated to 10°C or 20°C for 24 days. Five individuals from each group were placed in each of eight 40-ml centrifuge tubes closed with porous foam plugs and placed in a block heater. Temperature was then raised from room temperature (22°C) 1 degree every 5 min. After a temperature of 41°C was reached, and at each

subsequent 1°C increase in temperature up to 49°C, one tube each of 10° and 20°C acclimated snails was removed. All snails were allowed 24 h to recover. The number of snails reattached to walls of each tube then were recorded as alive, while those not attached were recorded as dead.

Respiratory response to temperature

Oxygen consumption rates were determined with Clark-type electrodes (YSI Model-53 polarographic silver-platinum oxygen electrodes) used in chambers modified with a glass annulus (Burky, 1977), utilizing the methods for small gastropods described in McMahon (1973) and McMahon and Russell-Hunter (1977, 1978). Respiration chambers were maintained at constant temperature ($\pm 0.05^\circ\text{C}$) with a Haake type-Fe constant temperature circulator. A Honeywell Elektronik-16 strip chart recorder was used to monitor changes in chamber oxygen concentration. All oxygen consumption rates were determined for subsamples chosen to have shell lengths within ± 2.5 mm of a chosen median value (numbers of individuals in each subsample depending on median shell length). In general, subsamples for each set of experiments were representative of the size range of adult snails in the Little Sippewisset population.

Six subsamples of snails acclimated to 10°C or to 20°C for 20–40 days were used to determine aquatic respiratory response to increasing temperature at three salinities (100% and 50% MBL seawater, and tap-water). Each subsample was placed in the chamber with 4 ml of the appropriate medium at the temperature of acclimation, with a small amount of streptomycin added to inhibit bacterial growth. The respiration chamber temperature was then lowered slowly (1°C/5 min) to 5°C. The oxygen probe was temperature equilibrated in a blank chamber, with the appropriate medium plus streptomycin but without snails (none of the blanks showed significant O₂ consumption). After this stabilization in the blank chamber, the probe was placed in the experimental chamber. Oxygen uptake rate of each subsample was then continuously monitored from near full air saturation with oxygen (PO₂ ca. 140–160 torr) over the first 10% decrease in O₂ tension, or for 1 h if less than 10% had been utilized. This procedure was repeated at 5°C intervals from 5–50°C, to above the upper lethal temperature.

Aerial oxygen consumption rates were measured by modifying the YSI oxygen probe with a neoprene O-ring to form an airtight seal against the walls of the chamber, and by running a 1.0 mm diameter plastic vent tube through the plastic plunger of the electrode (after McMahon and Russell-Hunter, 1977). These modifications allowed insertion of the probe into the chambers to form enclosed volumes of 2 ml. Six subsamples from each of the 10° and 20°C acclimation groups were placed in the chamber with a granule of silica-gel drying agent to prevent moisture condensation on the electrode membrane. The temperature then was gradually lowered from each acclimation temperature to 5°C. The electrode was temperature equilibrated with the vent tube open for 25 min. The probe then was placed in the experimental chamber for 10 min, with the vent tube open. The vent was then sealed and the change in oxygen concentration monitored for 1 h, or until 10% of the oxygen in the chamber was consumed. These measurements were repeated in 5°C intervals at increasingly higher temperatures to 50°C. At the end of each set of determinations, the volume of the experimental individuals and of the silica-gel granule were measured by fluid displacement. By subtraction of their volume from the initial 2 ml chamber volume, the experimental air volume was determined.

Respiratory response to hypoxia

The respiratory response of *Melampus* to hypoxia was determined by methods previously utilized for small molluscs (McMahon, 1972, 1973; McMahon and Russell-Hunter, 1978). The oxygen consumption rates of six subsamples of snails from each of the 10° and 20°C acclimation groups were monitored continuously with declining oxygen concentration in full strength MBL seawater (salinity = 31‰) at 20°C from near full air saturation (PO_2 ca. 140–160 torr) to an oxygen tension at which all uptake ceased. The method of measurement was as described previously for determinations of oxygen consumption rates at full air saturation except that monitoring continued through the entire range of oxygen concentrations over which any uptake occurred. Oxygen uptake rates for each subsample were computed for each 10% decrease in oxygen concentration from near full air saturation with oxygen until an oxygen tension of 30% of air saturation was reached. Thereafter uptake rates were determined for every 5% decrease in oxygen tension until consumption ceased at <3% of air saturation with oxygen ($PO_2 < 4.8$ torr).

Posthypoxic respiratory response in *Melampus* was determined by methods described for other small gastropods (McMahon, 1973; McMahon and Russell-Hunter, 1978). The oxygen uptake of two groups of snails acclimated to 20°C for 49 days was monitored at 20°C from near full air saturation at 20°C in full strength MBL seawater to an oxygen tension at which oxygen uptake ceased. The subsamples were then maintained at this oxygen tension (<3% of air saturation, $PO_2 < 4.8$ torr) for 5 h, after which the chamber water was fully reaerated. Thereafter, the two subsamples were treated differently. The first was used to determine the occurrence of any oxygen debt repayment. Oxygen consumption rates were monitored at near full air saturation immediately after reaeration, at 20 min intervals for 2 h, at 1 h intervals for 4 h, and then at 2–3 h intervals for the remaining 22 h after aeration. After this 28 h period at full air saturation, the oxygen uptake rates of this subsample were again determined over the full range of oxygen concentrations from near air saturation to the oxygen tension at which uptake ceased.

The second subsample was also continuously monitored for O_2 uptake from full air saturation with oxygen to the tension at which uptake ceased, but this was done immediately upon reaeration after 5 h of hypoxia. After this return to full air saturation with oxygen, oxygen consumption rates were measured with declining oxygen concentration at 5 h intervals, for a 25 h period after the initial 5 h period of hypoxia.

Diurnal and tidal influences on oxygen consumption

Diurnal and tidal influences on oxygen consumption of *Melampus* were investigated using two groups of snails. The first was collected in the field at Little Sippewisset during a set of peak spring tides on 9 August 1975 and returned to the laboratory for immediate experiment. The second group had been collected from Little Sippewisset on 9 June 1975 and maintained in the laboratory without tidal influences for 46 days at 20°C ($\pm 0.5^\circ\text{C}$) and on a 16L:8D cycle before testing began along with the freshly collected group.

The experimental procedure was the same for both groups. Three subsamples of individuals with similar shell lengths were selected from each group. Their aerial oxygen uptake rates were determined as described previously, at approximately 1 h intervals over at least a 24 h period beginning at 0900 h. The two groups were so tested within 3 days of each other.

Computation of oxygen uptake rates

For all determinations of oxygen consumption, uptake rates were expressed as mean $\mu\text{l O}_2/(\text{animal} \cdot \text{h})$ for each subsample. After each determination, the individuals in the subsample were blotted on filter paper and wet weighed to the nearest 0.1 mg. The mineral component of the shell was then removed by dissolution in 10% (by volume) nitric acid (after McMahon, 1973, 1975). The remaining flesh was then carefully separated from the periostracal component of the shell and dried to constant weight in a 100°C drying oven (>24 h at 100°C). The mean per animal oxygen consumption rates of each subsample were then divided by their mean dry flesh weight to give weight specific uptake rates [$(\mu\text{l O}_2/(\text{mg} \cdot \text{h})$ or $\dot{V}\text{O}_2$].

RESULTS

Submergence tolerance

Temperature had a more pronounced effect on tolerance of submergence than did salinity (Table I). Specimens of *Melampus* at 20°C survived only 1 day in tap-water, 2 days in 100%, 50%, and 25% seawater, and 3 days in 75% seawater. Those maintained at 10°C survived 8 days in tap-water and up to 14 days in 100% to 25% seawater (Table I).

Effect of salinity on thermal tolerance

The heat coma temperature (HCT) of *Melampus* did not show significant correlation with size measured as shell length ($r = 0.36$, $P > 0.1$, $N = 25$). Therefore, mean values of HCT with their SDs, SEs, and 95% confidence intervals were computed. *Melampus* appears to be able to make substantial acclimatory adjustments in HCT to compensate for sustained shifts in ambient temperature. The mean HCT of freshly collected specimens (ambient air temperature = 17°C) in full strength seawater was 38.8°C ($\text{SD} = \pm 1.12^\circ\text{C}$, $N = 25$) (Fig. 1). After 12 days at 20°C this value rose to 40.8°C ($\text{SD} = \pm 1.51$, $N = 20$) after which no significant change ($P > 0.05$) in mean HCT occurred. After 12 days at 10°C mean HCT fell to 36.3°C ($\text{SD} = \pm 1.75^\circ\text{C}$, $N = 20$) after which no significant variation

TABLE I

Effects of temperature and salinity on the survival of Melampus bidentatus during prolonged submergence.

Days submerged	Per cent surviving									
	10°C Acclimated					20°C Acclimated				
	100% SW	75% SW	50% SW	25% SW	Tap- water	100% SW	75% SW	50% SW	25% SW	Tap- water
1	100	100	100	100	100	100	100	100	100	100
2	90	100	100	100	100	100	100	100	100	0
3	100	100	100	100	100	0	100	0	0	0
4	100	100	100	100	90	0	0	0	0	0
6	100	100	100	100	90	0	0	0	0	0
8	100	90	90	100	90	0	0	0	0	0
10	100	100	100	40	0	0	0	0	0	0
14	80	90	100	90	0	0	0	0	0	0

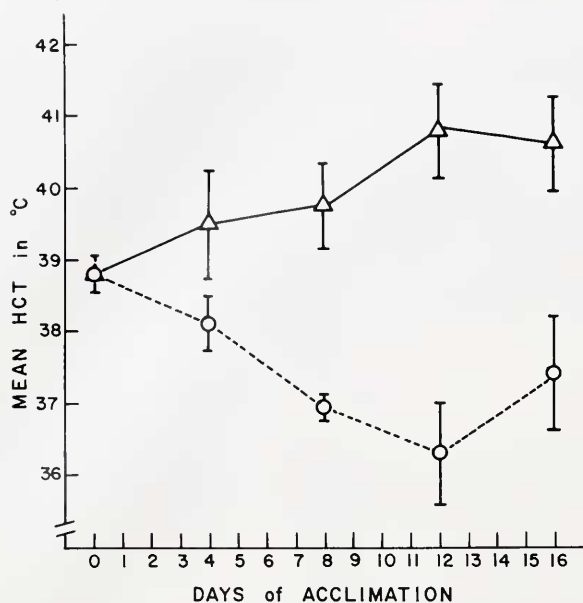


FIGURE 1. Heat coma temperature (HCT) in *Melampus bidentatus*, and its acclimation with time. The vertical axis shows mean HCT in degrees centigrade. The horizontal axis represents the duration of the temperature acclimation period in days. All snails were freshly collected from the Little Sippiswisset Marsh at day zero (ambient air temperature = 17°C). The open circles connected by dashed lines are mean HCT values of snails held at 10°C, and the open triangles connected by solid lines, mean HCT values of similar snails held at 20°C. Each point is the mean value for 20–30 individuals, and the vertical bars represent 95% confidence limits about mean HCT values.

in HCT ($P > 0.05$) occurred (Fig. 1). Therefore, acclimation periods greater than 20 days were used for all subsequent tests of temperature tolerance and of oxygen consumption. Mean HCT values of 20°C-acclimated snails were significantly higher ($T \leq 2.227$, $P < 0.05$) than those acclimated to 10°C, averaging 4.93°C higher over all salinities tested ($SD = \pm 2.62$, range = 3.01–9.45, $N = 5$). This yields a rate of HCT acclimation per degree increase in acclimation temperature of 0.49°C (Table II).

A one-way analysis of variance revealed that salinity also had a highly significant effect on HCT in *Melampus* at both acclimation temperatures (10°C accli-

TABLE II

Mean heat coma temperature (HCT), standard deviation (SD), standard error (SE), 95% confidence limit (95%) and sample size (N) in relation to salinity and temperature in *Melampus bidentatus*.

Salinity	10°C Acclimated					20°C Acclimated				
	HCT (°C)	SD	SE	95%	N	HCT (°C)	SD	SE	95%	N
100% SW	37.44	±1.62	±0.38	±0.75	18	40.65	±1.53	±0.35	±0.67	20
75% SW	35.26	±2.47	±0.57	±1.11	19	39.90	±1.25	±0.28	±0.55	20
50% SW	35.43	±1.74	±0.47	±0.91	14	39.75	±1.89	±0.42	±0.83	20
25% SW	31.30	±3.54	±0.79	±1.55	20	40.75	±2.71	±0.61	±1.19	20
Tap-water	32.20	±4.65	±1.04	±2.04	20	35.21	±3.52	±0.81	±1.58	19

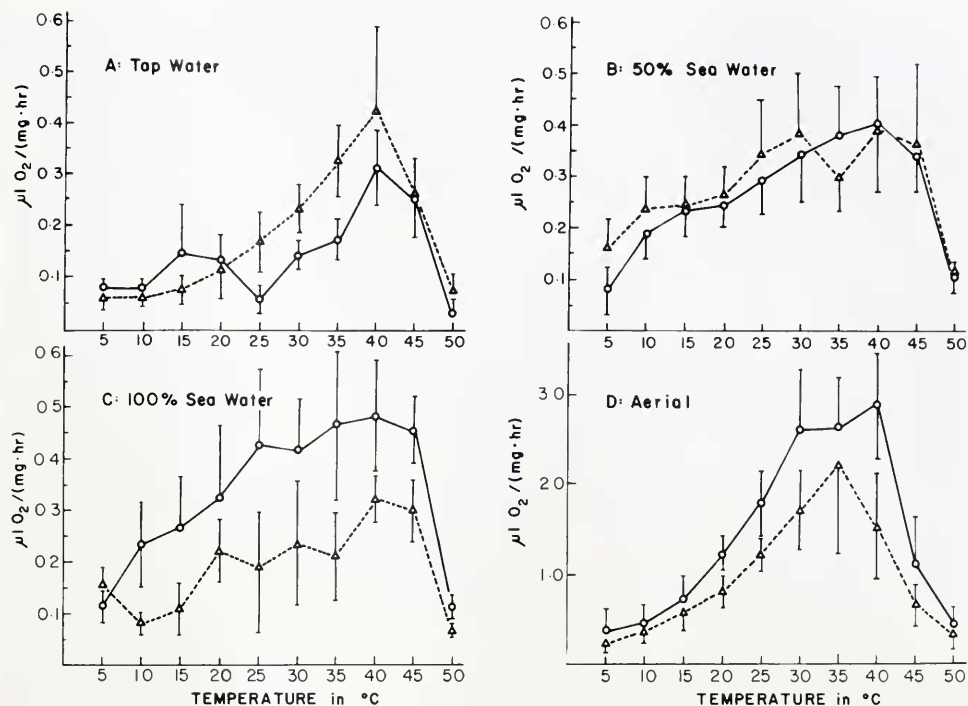


FIGURE 2. Temperature and salinity effects on aerial and aquatic weight-specific oxygen consumption rates ($\dot{V}O_2$) in *Melampus bidentatus*. Open circles connected by solid lines, mean $\dot{V}O_2$ [in $\mu\text{l O}_2 / (\text{mg dry tissue wt} \cdot \text{h})$ at STP] of 20°C-acclimated individuals and open triangles connected by dashed lines, mean $\dot{V}O_2$ of 10°C-acclimated individuals. A, Mean $\dot{V}O_2$ in tap-water. B, Mean $\dot{V}O_2$ in 50% seawater (salinity = 15.5‰). C, Mean $\dot{V}O_2$ in 100% seawater (salinity = 31.0‰). D, Mean $\dot{V}O_2$ in air (note difference in scale of the vertical axis). The vertical bars represent 95% confidence limits about the mean $\dot{V}O_2$ values for six replicate subsamples (limits not indicated if they overlap the mean $\dot{V}O_2$ value of the other acclimation group at the same temperature).

mated, $F_{4,86} = 12.25$, $P < 0.001$; 20°C acclimated, $F_{4,94} = 18.96$, $P < 0.001$). A Student-Newman-Keuls test revealed that mean HCT in 10°C-acclimated individuals in 100% seawater was significantly greater ($P < 0.05$) than mean HCT in all other aquatic media. Mean HCT values in tap-water and 25% seawater did not differ significantly ($P > 0.05$), nor did values for 50% and 75% seawater. In contrast, HCT values for 50% and 75% seawater were significantly greater ($P < 0.05$) than the values for 25% seawater and tap-water. For 20°C-acclimated individuals mean HCT in tap-water was significantly less ($P < 0.05$) than those in salinities between 25% and 100% seawater, which were not significantly different ($P > 0.05$) from each other.

The $LD(T)_{50}$ of *Melampus* in air was 44.5°C for 10°C-acclimated individuals and 44.7°C for those acclimated to 20°C. All 10°C-acclimated snails survived at 44°C in air but none at 45°C. Those acclimated to 20°C died at either 45°C or 46°C in air with none surviving at 47°C.

Respiratory response to temperature and salinity

Figure 2 shows the change in mean weight-specific oxygen rate in $\mu\text{l O}_2 / (\text{mg} \cdot \text{h})$ ($=\dot{V}O_2$) of 10°- and 20°C-acclimated snails under differing conditions of tem-

perature and salinity. Least squares linear regression analysis of logarithmic transformations of values in $\mu\text{l O}_2/(\text{animal}\cdot\text{h})$ versus mean dry weight values revealed that only 9 out of 80 such determinations showed any significant ($P < 0.05$) correlation between dry tissue weight and oxygen uptake rate. (In part, this was due to the limited size range of adult snails used from in the Little Sippewisset population.) Therefore, all oxygen consumption rates were computed as means combining all repeated individual determinations.

A two-way analysis of variance of the effect of test temperature and acclimation temperature on mean $\dot{V}\text{O}_2$ revealed that test temperature had a significant effect ($F_{9,27} > 4.8$, $P < 0.01$) in all four media tested. Acclimation temperature only affected oxygen uptake rates significantly in 100% seawater ($F_{1,9} = 12.8$, $P < 0.001$) and in air ($F_{1,9} = 10.5$, $P < 0.01$). Further analysis by t test of the mean $\dot{V}\text{O}_2$ values of the 10°- and 20°C-acclimated snails at each salinity-temperature combination tested revealed that significant differences ($P < 0.05$) occurred only at 25°, 30° and 35°C in tap-water, only at 5°C in 50% seawater, at 5°, 10°, 15°, 25°, 35°, 40°, 45°, and 50°C in 100% seawater and at 20°, 25°, 30°, and 40°C in air. Figures 2c and d show that the oxygen uptake rates of 20°C-acclimated individuals (both in 100% seawater and air) were always higher than those of 10°C-acclimated individuals, except at 5°C in 100% SW. Such elevation of rates in individuals acclimated to higher temperatures has been referred to as "reverse" or "paradoxical" acclimation (Berg, 1951, 1952, 1953; Proser, 1973).

A two-way analysis of variance of the effect of medium (aerial vs. aquatic) and test temperature on mean $\dot{V}\text{O}_2$ revealed that the medium significantly affected oxygen consumption rate ($F_{3,27} > 15.0$, $P < 0.001$). In both 10°- and 20°C-acclimated snails, $\dot{V}\text{O}_2$ was not significantly different ($P > 0.05$) among all three aquatic media, but was significantly greater in air ($P < 0.05$) than in the aquatic media (as analyzed by Tukey's test for multiple pair-wise comparisons).

Table III shows values of Q_{10} corresponding to changes in $\dot{V}\text{O}_2$ over 5°C intervals for each of the four test media (100% and 50% seawater, tap-water and air). A two-way analysis of variance comparing the effects of temperature (over the series of 5°C intervals) and medium showed that while temperature significantly affected Q_{10} ($F_{8,24} > 2.58$, $P < 0.05$) in both 10° and 20°C acclimation groups, the

TABLE III

Mean Q_{10} values for the change in the mean weight specific oxygen consumption in individuals of Melampus bidentatus (acclimated to 10°C or 20°C) over 5°C intervals from 5°C to 50°C in 100% and 50% sea water, tap water, and in air.

Temperature range in °C	10°C Acclimated				20°C Acclimated			
	100% SW	50% SW	Tap-water	Air	100% SW	50% SW	Tap-water	Air
5-10	0.253	2.086	1.000	2.527	4.291	5.724	0.976	1.426
10-15	1.834	1.070	1.552	2.504	1.295	1.936	3.294	2.640
15-20	4.227	1.199	2.250	1.944	1.479	0.854	0.819	2.943
20-25	0.734	1.606	2.224	2.293	1.739	1.444	0.647	2.136
25-30	1.533	1.254	1.895	1.945	0.954	1.372	1.862	2.143
30-35	0.805	0.601	1.989	1.716	1.249	1.235	1.453	1.018
35-40	2.322	1.733	1.651	0.464	1.070	1.125	3.203	1.190
40-45	0.873	0.843	0.359	0.186	0.887	0.703	0.640	0.148
45-50	0.093	0.092	0.085	0.234	0.056	0.102	0.013	0.152

type of medium did not ($F_{3,24} < 0.98$, $P > 0.1$). Similarly, a two-way analysis of variance of the effects of test temperature and acclimation temperature on Q_{10} revealed that acclimation temperature did not significantly affect the respiratory response to increasing temperature in any of the four media tested ($F < 1.08$, $P > 0.1$). This result is supported by the lack of any significant difference ($P < 0.05$) between mean Q_{10} values of 10°- and 20°C-acclimated individuals for any of the pairs of 36 medium-test temperature combinations (as displayed in Table III) analyzed.

The mean sequential Q_{10} values for 5°C intervals throughout the normal 10°–40°C ambient temperature range of *Melampus* were: 1.909 (SD = ± 1.288) in 100% seawater; 1.244 (SD = ± 0.404) in 50% seawater; 1.937 (SD = ± 0.288) in tap-water; and 1.813 (SD = ± 0.718) in air for 10°C-acclimated individuals. Corresponding values for 20°C-acclimated snails were: 1.301 (SD = ± 0.285) in 100% seawater; 1.328 (SD = ± 0.363) in 50% seawater; 1.725 (SD = ± 1.210) in tap-water; and 2.016 (SD = ± 0.769) in air. In air, the $\dot{V}O_2$ of 10°C-acclimated snails generally increased with temperature to 35°C. Above this, the rate declined due to temperature stress. In 20°C-acclimated individuals, $\dot{V}O_2$ increased to 25°C, remained stable through 40°C (Q_{10} between 25° and 40°C = 1.374), and then declined (Fig. 2d). Similar regulation of $\dot{V}O_2$ with increasing temperature between 25° and 45°C ($Q_{10} = 1.03$) is very apparent in 20°C-acclimated snails in 100% seawater (Fig. 2c).

Respiratory response to hypoxia

Figure 3 shows the mean $\dot{V}O_2$ values ($n = 6$) of 10°- and 20°C-acclimated individuals with decreasing O_2 concentration in 100% seawater at 20°C. $\dot{V}O_2$ values with decreasing oxygen concentration for each of six individual subsamples from the 10°C or 20°C acclimation groups were expressed as fractions of the rate at

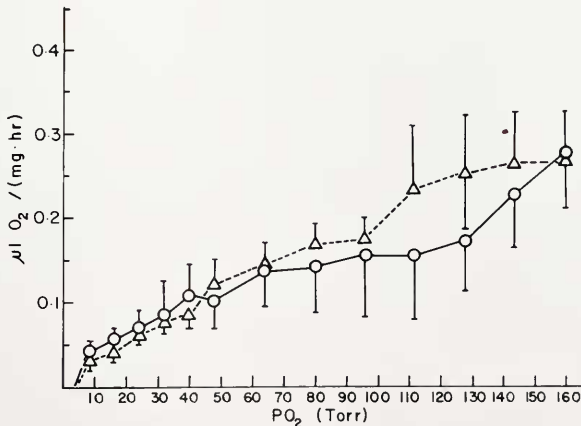


FIGURE 3. Effect of declining oxygen concentration on mean weight-specific oxygen consumption rate ($\dot{V}O_2$) of *Melampus bidentatus* in seawater (salinity = 31.0‰), from full air saturation with oxygen ($PO_2 = 159$ torr). The horizontal axis represents the partial pressure of oxygen in torr and the vertical axis, mean $\dot{V}O_2$ [in $\mu l O_2 / (mg \text{ dry tissue wt} \cdot h)$ at STP]. The open circles connected by solid lines are $\dot{V}O_2$ values of 20°C-acclimated individuals and the open triangles connected by dashed lines, $\dot{V}O_2$ values of 10°C-acclimated individuals. The vertical bars represent 95% confidence limits about the mean $\dot{V}O_2$ values for six replicate subsamples (limits not indicated if they overlap the mean $\dot{V}O_2$ value of the other acclimation group at the same temperature).

air saturation with oxygen and fitted versus PO_2 in torr to a quadratic equation. The resultant quadratic coefficient (b_2) is a predictor of the degree of regulation (independence) of oxygen uptake with declining oxygen concentration. It becomes an increasingly large negative value as the degree of regulation increases (Mangum and Van Winkle, 1973). Expressed as $b_2 \times 10^3$, the means of these values for 10°C-acclimated ($b_2 \times 10^3 = -0.0183$, $SD = \pm 0.0098$, $N = 6$) and 20°C-acclimated individuals ($b_2 \times 10^3 = -0.0067$, $SD = \pm 0.0337$, $N = 6$) were not significantly different ($T = 0.817$, $P > 0.1$). Adjusted $\dot{V}O_2$ values of all six subsamples were fitted to a single quadratic equation, to yield $b_2 \times 10^3$ values of -0.0175 and -0.004 for the 10°- and 20°C-acclimated groups, respectively. When based on the mean values presented in Fig. 3, $b_2 \times 10^3$ values were -0.0174 (10°C acclimated) and -0.0021 (20°C acclimated). As such, all three methods of computation of average $b_2 \times 10^3$ values yielded similar results, with values ranging between -0.0021 and -0.0183 . This range of values indicated that *Melampus* was unable to regulate oxygen consumption with decreasing oxygen concentration whether acclimated to 10° or 20°C (Fig. 3).

After exposure to hypoxic conditions, *Melampus* appeared to pay an oxygen debt. Figure 4a reveals that $\dot{V}O_2$ at near full air saturation with oxygen ($PO_2 > 150$ torr) increased to 2.4 times the prehypoxic rate, and remained noticeably elevated for about 2 h after return to full air saturation following 5 h of low oxygen stress. The amount of debt paid, as a volume of oxygen respired, was estimated after the method of McMahon and Russell-Hunter (1978). The portion of each posthypoxic $\dot{V}O_2$ value that represented an increase over the prehypoxic rate of $0.213 \mu l O_2/(mg \cdot h)$ was multiplied by each time span (as fractions of an h) extending from halfway between each measurement and the preceding one to halfway between the measurement and the next, until $\dot{V}O_2$ again fell close to the prehypoxic value. Total oxygen debt was estimated as the volume of oxygen that would have been respired at the prehypoxic rate over the 5 h period of hypoxia. Such computations showed that total debt repayment in *Melampus* was only about 29.5% of the estimated accumulated oxygen debt. After returning to prehypoxic rates at 2 h posthypoxia, $\dot{V}O_2$ again slowly increased reaching $0.616 \mu l O_2/(mg \cdot h)$ by 28 h after the low oxygen stress period (Fig. 4a). Figure 4b shows the $\dot{V}O_{2s}$ with decreasing oxygen concentration of a subsample of *Melampus* before and after 5 h of hypoxia. Before hypoxic stress $\dot{V}O_2$ was not regulated; it decreased proportionately with oxygen concentration (Fig. 3, 4b). A similar response occurred just after return to air saturation from the 5 h of hypoxia, except that uptake rates were more than twice prehypoxic levels, due to payment of the oxygen debt just described. This lack of regulation is reflected by high $b_2 \times 10^3$ values of -0.0176 before and -0.0030 just after the low oxygen stress period. However, from 5–20 h after hypoxia, oxygen consumption not only remained 30%–50% greater than prestress levels at PO_{2s} greater than 100 torr, but shifted markedly toward increased regulation. The rate of decline in $\dot{V}O_2$ was greatly reduced from full air saturation down to a PO_2 of 35 torr, the critical oxygen tension below which $\dot{V}O_2$ declined acutely with further decrease in PO_2 (Fig. 4b). This increase in the posthypoxic regulatory ability of *Melampus* is manifested by a sizable decrease in $b_2 \times 10^3$ values (5 h posthypoxia, $b_2 \times 10^3 = -0.0500$, 10 h = -0.0014 , 15 h = -0.0245 , and 20 h = -0.0576) averaging -0.0332 . This indicates a shift to moderate or good regulation of oxygen uptake with declining oxygen tension (Mangum and Van Winkle, 1973).

This combination, a general increase in $\dot{V}O_2$ over the full range of PO_2 from air saturation and a shift towards increased regulation of oxygen consumption,

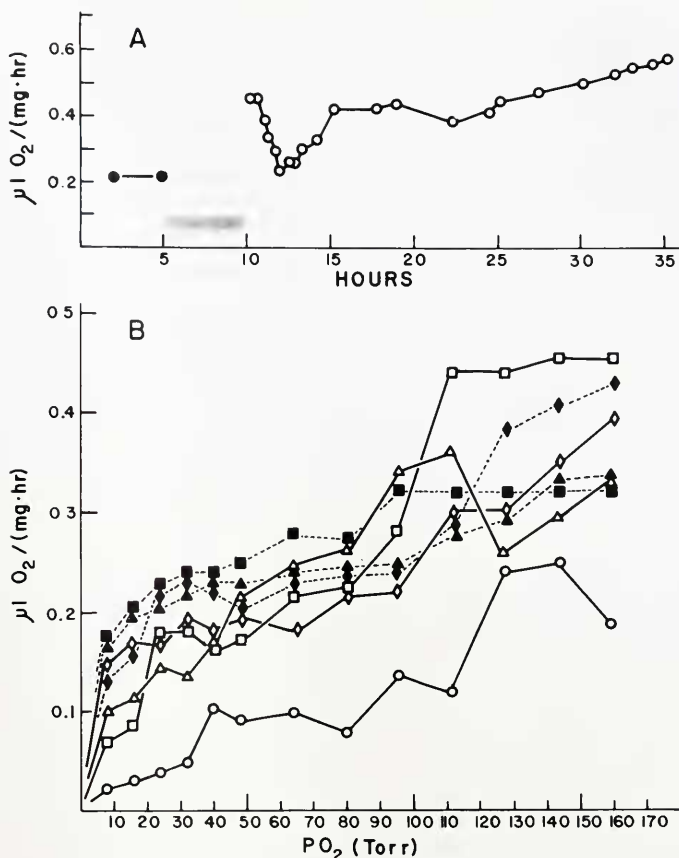


FIGURE 4. The effect of a 5 h period of anoxia on the weight-specific oxygen consumption rate [$\dot{V}O_2$ in $\mu l O_2 / (mg \text{ dry tissue wt} \cdot h)$ at STP] of *Melampus bidentatus* in 100% seawater at 20°C. A, The effect of anoxia on $\dot{V}O_2$ in fully air-saturated seawater. The horizontal axis is time in hours and the vertical axis $\dot{V}O_2$ as above. The solid circles represent $\dot{V}O_2$ before 5 h of anoxia; and the open circles, $\dot{V}O_2$ for up to 25 h after return to fully air-saturated seawater ($PO_2 = 159$ torr) from the 5 h of anoxia. B, Effect of five hours of anoxia on $\dot{V}O_2$ with decreasing oxygen concentration from full air saturation ($PO_2 = 159$ torr). Horizontal axis, partial pressure of oxygen in torr; vertical axis, $\dot{V}O_2$ as above. Open circles connected by solid lines represent $\dot{V}O_2$ with declining oxygen concentrations before anoxia. Open triangles, squares, and diamonds connected by solid lines represent $\dot{V}O_2$ immediately after, 5 h after and 10 h after return to fully air-saturated seawater from anoxia, respectively. Solid triangles, squares and diamonds connected by dashed lines represent $\dot{V}O_2$ 15 h, 20 h, and 25 h after anoxia, respectively.

allows *Melampus* to maintain oxygen uptake rates at or near prehypoxic rates at full air saturation, down to PO_{2s} as low as 30 torr for 5 to 20 h after 5 h of hypoxia (Fig. 4b). In untreated snails at 30 torr, there is no regulation, and uptake rates run at about 20–40% of poststress rates. By 25 h posthypoxia, the ability of *Melampus* to regulate oxygen consumption appears to be lost ($b_2 \times 10^3 = -0.0012$), but $\dot{V}O_2$ remains elevated over the full range of oxygen tensions tested, allowing continued maintenance of uptake rates close to prestress full air saturation levels at PO_{2s} as low as 65 torr. In the first subsample, $b_2 \times 10^3$ values remained low and uptake rates elevated after 28 h return from hypoxia at -0.0426 . This indicated slightly longer retention of oxygen regulation in this group.

Diurnal and tidal influence on oxygen consumption

The repeated determinations of aerial $\dot{V}O_2$ at 20°C of three subsamples of snails freshly collected from the field, and of those maintained at 20°C in the laboratory on a L:D cycle of 16:8, without tidal influence, were averaged on an hourly basis, and then computed as a running smoothed mean of the average hourly value and their two adjacent values. These smoothed values were then expressed as percentages of the overall mean $\dot{V}O_2$ of each group and plotted against the average time of measurement for each original hourly triplet of determinations (Fig. 5). In freshly collected snails, aerial $\dot{V}O_2$ increased steadily from a low value of 88% of the mean at 1200 h (Eastern Standard Time) to a peak $\dot{V}O_2$ of 117% of the mean at 2359 h. This was followed by decline to 83% to 84% of the mean at 1200 h the following day, after which uptake again began to rise (Fig. 5). The diurnal pattern of $\dot{V}O_2$ variation was apparently reversed in the laboratory-maintained specimens. Peak rates of 125%–129% of mean $\dot{V}O_2$ occurred at 1000–1600 h and then declined to 72%–74% of the mean at 2300–0300 h (the middle of the artificial 8-h dark period). $\dot{V}O_2$ thereafter rose steadily, returning to near the mean value by 1200 h the following day (Fig. 5).

DISCUSSION

These results clearly indicate that, in such aspects of its somatic physiology as respiration and water metabolism, *Melampus bidentatus* is highly adapted to life

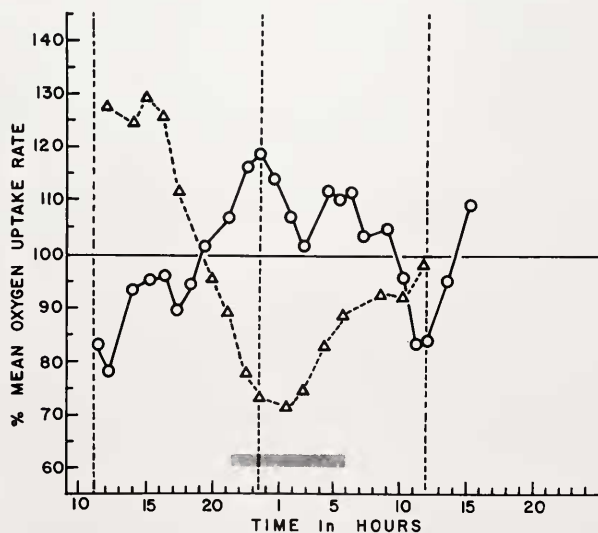


FIGURE 5. Diurnal variation in weight-specific oxygen consumption rate ($\dot{V}O_2$) of *Melampus bidentatus*. Horizontal axis, time in hours from 1000 h, when $\dot{V}O_2$ measurement began, until 1600 h the following day, when the experiment ended. The vertical axis, the per cent value by which smoothed mean values of $\dot{V}O_2$ differ from the mean value of $\dot{V}O_2$ over the entire time course of the experiment (represented by the solid line extending horizontally from the 100% point on the vertical axis). Open circles connected by solid lines, smoothed values of $\dot{V}O_2$ computed from three subsamples of similar sized individuals freshly collected from the Little Sippiswisset Marsh. Open triangles connected by dashed lines, similar smoothed values of $\dot{V}O_2$ for individuals maintained at 20°C and at 16L:8D for 46 days in the laboratory. The shaded horizontal bar indicates the duration of the dark period to which laboratory-maintained individuals were exposed and the dashed vertical lines, the times of peak high tides in the Little Sippiswisset Marsh during the experiment.

in the upper levels of the littoral salt-marsh environment. A primitive pulmonate of the family Ellobiidae, it has lost the ctenidium associated with aquatic respiration and modified the mantle-cavity into a highly vascularized diffusion lung for aerial gas exchange (Apley, 1967, 1970; Russell-Hunter *et al.*, 1972; Russell-Hunter, 1979; Price, 1976, 1980). This represents adaptation to the high degree of aerial exposure in the upper reaches of the salt-marsh, since it is exposed to air over 95% of the lunar tidal cycle, and is submerged only at peak spring high tides (Apley, 1967, 1970; Russell-Hunter *et al.*, 1972; McMahon and Russell-Hunter, 1977; Price, 1976, 1980). Without a gill, the snail's respiratory gas exchange when submerged is cutaneous over its general body surface. It does not appear to use a gas bubble in the mantle-cavity as a physical gill (Russell-Hunter *et al.*, 1972). Submerged oxygen uptake is less than corresponding aerial oxygen uptake at temperatures between 5°C and 45°C (Fig. 2).

It has been proposed that *M. bidentatus*, as a lung-breather, would asphyxiate during even relatively short periods of submergence (Russell-Hunter *et al.*, 1972). The present results indicate that *M. bidentatus* is more tolerant of submergence than previously suspected, surviving (at 20°C) at least 2 days in 100% to 25% SW (3 days in 75% SW) and at least 1 day in tap-water (Table I). Price (1979) reported even greater tolerances, of more than 10 days submerged in 100% SW and up to 3 days in tap-water at 20°C. These periods are far longer than the 1.5–3 h of submergence *M. bidentatus* is reported to experience at high spring tides (Apley, 1967; Russell-Hunter *et al.*, 1972; McMahon and Russell-Hunter, 1977), and may represent a sort of "safety factor" evolved to allow survival under even the most extreme flooding by storm tides or prolonged rainfall. *Amphibola crenata*, a related pulmonate endemic to estuarine mud-flats in New Zealand, appears to be much better adapted to aquatic conditions than *M. bidentatus*. It survives 14 days in freshwater and up to 30 days in seawater, but only 1 day without water (Farnie, 1919), while *M. bidentatus* can survive for at least 3 days in all relative humidities above 50% (Price, 1980).

At 10°C, tolerance of submergence (in 25%–100% SW) is remarkably increased in *M. bidentatus*; 80% to 100% survived on termination of the experiment after 14 days of submergence in seawater and near 100% survived after 8 days in tap-water (Table I). At such lower temperatures (and in salinities above 50% SW), oxygen uptake is reduced by a combination of low temperature effects and respiratory "reverse" acclimation. The O₂ uptake rates of submerged individuals at 10°C are only 25% to 75% of individuals at 20°C (Figs. 2a, b, c). Reduction in oxygen demand could allow individuals submerged at 10°C to survive indefinitely by cutaneous gas exchange in all salinities with no severe osmotic stress.

Melampus bidentatus overwinters by burrowing into the marsh floor or by entering the abandoned burrows of the fiddler crab, *Uca*, (Hausman, 1932; Apley, 1967, 1970; Price, 1976, 1980) to soil depths that remain unfrozen. When covered by spring tides, *Uca* burrows and the peaty marsh soils remain flooded far longer than the marsh surface. During winter months *M. bidentatus* is inactive (Apley, 1967, 1970; Russell-Hunter *et al.*, 1972; Price, 1976, 1980), and thus can be exposed to rather long periods of submergence in waters of relatively low ambient temperature. Reduced metabolic rates in cold-acclimated individuals allow survival of much longer periods of submergence with entirely cutaneous gas exchange. During spring and summer *M. bidentatus* returns to the marsh surface and can actively avoid submergence during spring tides by crawling up the stems of marsh grass (Apley, 1967, 1970; Russell-Hunter *et al.*, 1972; Price, 1976, 1980).

M. bidentatus spends a portion of its active hours on the high salt-marsh surface

and is often directly exposed to sunlight. Such insolation can cause salt-marsh surface temperatures to be several degrees higher than ambient air temperatures. *M. bidentatus* can tolerate high temperatures, as can certain other gastropods of the higher intertidal (Gowanloch and Hayes, 1926; Broekhuysen, 1940; Evans, 1948; Fraenkel, 1961, 1966, 1968; Lewis, 1963; Sandison, 1967; McMahon and Russell-Hunter, 1977). The temperatures at which activity ceased (HCT) in 20°C-acclimated specimens of *M. bidentatus* ranged between 40.7°C in 100% SW and 35.2°C in tap-water. For 10°C-acclimated individuals, the corresponding range was lower (Table II). This decrease in tolerance would suggest that thermal tolerance of submerged individuals is subject to temperature acclimation. In contrast, in air, temperature acclimation did not affect lethal temperatures for *M. bidentatus*. The LD(T)₅₀'s of 10°C- and 20°C-acclimated individuals were very similar: 44.5°C and 44.7°C, respectively.

It appears that 10°C-acclimated individuals have somewhat depressed upper lethal temperature limits when submerged. In contrast, 20°C-acclimated snails appear to have nearly equal upper lethal limits in air and water. The reasons for this difference are not entirely clear. Certainly the large amounts of body water which *M. bidentatus* is capable of losing under desiccation stress (Russell-Hunter and Meadows, 1965; Price, 1980) indicate some short-term evaporative cooling in air is possible as a passive response. But the more likely explanation lies in the more complex interactions involving metabolic rates and acclimation, reviewed for marine and estuarine animals by Kinné (1964, 1971), Newell (1976), and Lockwood (1976). There was no significant difference in the HCT of 20°C-acclimated specimens of *M. bidentatus* in 100% to 25% SW (Table II). Regulation of thermal tolerance over a range of salinities is highly adaptive for a high salt-marsh animal which is exposed to extreme variations of both temperature and salinity.

However, when *M. bidentatus* was winter- or cold-conditioned (10°C-acclimated), it lost its ability to modify thermal tolerance levels; HCT decreased proportionately with salinity (Table II). This loss of ability to modify thermal tolerance with changing salinity would not lead to stress over the range of ambient temperatures encountered (in burrows) during the overwintering period.

Overwintering individuals of *M. bidentatus* are also tolerant of freezing temperatures. They can be maintained at -12°C for 3 days with mortality rates ranging from 85.7% for individuals with shell lengths of less than 5 mm to only 26.7% for those with shell lengths greater than 7 mm (Price, 1980; see also Hilbish, 1981). Similar tolerance to freezing has been described in other intertidal molluscs.

Aquatic oxygen consumption of individuals of *M. bidentatus* acclimated at 10°C and 20°C is much less than corresponding aerial rates over all tested salinities and temperatures (Fig. 2), except for 20°C-acclimated individuals in tap-water (an "unnatural" condition poorly tolerated by *M. bidentatus*, see Table I).

It is obvious that a pulmonate like *M. bidentatus*, which has lost the ctenidium and evolved a highly vascularized mantle-cavity as an aerial diffusion lung, and which spends the vast majority of each lunar tidal cycle exposed to air, should show elevated rates of aerial $\dot{V}O_2$ (Fig. 2a-d). In contrast, the aquatic $\dot{V}O_2$ values of prosobranch littorinid snails which retain the ctenidium are generally reported to be greater than (or, at least, similar to) aerial rates of O_2 uptake. Such respiratory patterns have now been described for *Littorina neritoides*, *L. saxatilis* (more properly *L. rudis*), *L. littorea*, and *L. obtusata* (Toulmond, 1967a, b; Raffy, 1933; Fischer *et al.*, 1933; McMahon and Russell-Hunter, 1977). In more aquatic subtidal prosobranchs, aerial $\dot{V}O_2$ is generally much less than aquatic rates of O_2 uptake (McMahon and Russell-Hunter, 1977; McMahon, unpublished data). Because O_2

uptake and activity are predominately aerial in *M. bidentatus*, this species appears far better adapted to terrestrial life than marine high-littoral prosobranchs. A pattern of nearly equivalent aerial and aquatic $\dot{V}O_2$ is also retained by primitive high littoral estuarine pulmonate, *Amphibola crenata*, which is somewhat more aquatic than *M. bidentatus* (see discussion of submergence tolerance above). *Amphibola* is apparently able to maintain equivalent $\dot{V}O_2$ in air or in seawater (Shumway, 1981) but it may not have as great a capacity to tolerate extended periods of aerial exposure as does *M. bidentatus* (Farnie, 1919).

Salinity does not greatly effect $\dot{V}O_2$ in *M. bidentatus* over a range of 0% to 100% SW. *A. crenata* is similar in this respect (Shumway, 1981). Both Kinné (1971) and Schlieper (1971), in their extensive reviews of the effects of salinity on estuarine organisms, state that there are relatively few highly euryhaline species in which metabolism remains independent of salinity. Among prosobranch gastropods only two essentially freshwater species display any salinity independence in O_2 consumption: *Theodoxus fluviatilis* (Lumbye, 1958) and *Potamopyrgus jenkinsi* (Lumbye and Lumbye, 1965; Duncan, 1966; Duncan and Klekowski, 1967). For both *M. bidentatus* and *A. crenata*, such salinity independence of $\dot{V}O_2$ is highly adaptive for life in the highest reaches of salt-marshes or estuaries, where salinities vary acutely with both tidal cycle and rainfall (Lockwood, 1976; Kinné, 1971; Newell, 1976; Schlieper, 1971).

The aerial $\dot{V}O_2$ of 10°C-acclimated individuals of *M. bidentatus* displays a normal response (Prosser, 1973) to temperature, with Q_{10} values falling within the range of 1.5–2.5 (Table III, Fig. 2d). In contrast, aerial $\dot{V}O_2$ in 20°C-acclimated individuals appears to be temperature regulated, with little or no apparent increase in $\dot{V}O_2$ and with Q_{10} values near 1.0 between 30°C and 40°C (Table III, Fig. 2d). Similar regulation in aquatic O_2 consumption occurs at all tested temperature ranges in 50% and 100% SW (Table III, Fig. 2b, c). There is no apparent temperature regulation of aquatic O_2 consumption in either 10°C- or 20°C-acclimated individuals in tap-water (Table III, Fig. 2a). Such temperature regulation of $\dot{V}O_2$ within the normal ambient temperature and salinity range allows maintenance of relatively efficient metabolic rates in an intertidal environment characterized by acute, short-term temperature variations. Similar temperature regulation of $\dot{V}O_2$ has been reported for the intertidal prosobranchs, *Littorina littorea* (Newell, 1973; Newell and Pye, 1970a, b, 1971a, b; Newell and Roy, 1973; McMahon and Russell-Hunter, 1977), and *L. irrorata* (Shirley *et al.*, 1978), the intertidal bivalve, *Mytilus edulis* (Newell and Pye, 1970a, b), and in several other intertidal invertebrates (as reviewed by Newell, 1976, 1979).

In its respiratory schesis, *M. bidentatus* also displays the physiological phenomenon of "reverse" or "inverse" acclimation, whereby 20°C-acclimated snails have higher, *rather than the more normal lower*, values of $\dot{V}O_2$ than do snails acclimated to 10°C, at all test temperatures (for discussions of reverse acclimation see Burky, 1971; McMahon, 1972, 1973; Calow, 1975; Parry, 1978). such reverse respiratory acclimation to temperature has previously been reported in the intertidal limpet, *Patella vulgata* (Davies, 1965), a terrestrial stylommatophoran pulmonate, *Helix pomatia* (Blazka, 1954), four species of basommatophoran ancyliid limpets, *Ancylus fluviatilis*, *Acroloxus lacustris*, *Ferrissia rivularis*, and *Laevapex fuscus* (Berg, 1951, 1952, 1953; Berg *et al.*, 1958; Calow, 1975; Burky, 1969, 1970, 1971; McMahon, 1972, 1973, 1975). Other than molluscs, reverse acclimation has been reported in barnacles (Barnes *et al.*, 1963), a freshwater gammarid (Krog, 1954), a goldfish (Roberts, 1960, 1966), and a hylid frog, *Pseudocris triseriata* (Packard, 1972).

Many hypotheses have been made regarding the adaptive value of reverse acclimation. These include:

1. Reduction of $\dot{V}O_2$ in overwintering animals is an adaptation to hypoxic conditions during ice cover (Roberts, 1960, 1966; Krog, 1954);
2. The resultant decrease in metabolic rate conserves scarce energy stores in inactive (non-feeding) overwintering individuals (Burky, 1969, 1971; Packard, 1972);
3. The phenomenon is only one manifestation of a more general change in respiratory response involving a shift from oxygen dependence of $\dot{V}O_2$ in warm-acclimated individuals to strict regulation of $\dot{V}O_2$ in cold-acclimated individuals, allowing overwintering animals to maintain higher O_2 consumption rates than summer individuals in their normally hypoxic overwintering microhabitats (McMahon, 1973, 1975);
4. Metabolic rate is directly proportional to growth rates, and, therefore, slow growing overwintering individuals have a lower $\dot{V}O_2$ than fast growing summer individuals (Parry, 1978); and
5. Reverse acclimation and the large seasonal shifts in respiratory rate associated with it are a specific adaptation to the rapidly fluctuating temperatures associated with certain low temperature environments, such as streams and wave-swept littoral regions of lakes (Calow, 1975).

In *Melampus bidentatus*, overwintering individuals are inactive, do not feed, show little growth, and probably survive on accumulated energy stores (Apley, 1967, 1970; Russell-Hunter *et al.*, 1972). However, no direct data suggest tissue "degrowth" in *M. bidentatus*, as occurs in other pulmonates (Russell-Hunter and Eversole, 1976). In the marsh substratum, *M. bidentatus* encounters not only periodic submergence and low temperatures, but also periods of hypoxia resulting from decomposition of buried organic matter (Lockwood, 1976). Under these conditions, reverse acclimation would conserve overwintering energy stores (primarily glycogen in gastropods [Goddard and Martin, 1966]) at the same time reducing the demand for oxygen. Such a reduction is highly adaptive in these hypoxic overwintering conditions, and could be stabilized at levels that could be entirely supported by cutaneous respiration in such torpid overwintering individuals.

As case studies of poikilothermic organisms accumulate, it has become increasingly obvious that inverse or reverse acclimation evolved independently in a variety of species and phyletic groups in response to a series of quite different selective pressures. It cannot be described in terms of a single unified adaptive advantage for all the species in which it occurs, although its evolution may always have involved a reversal of pre-existing signals for genetically patterned control mechanisms. In *M. bidentatus*, reverse acclimation almost certainly involves a sort of "torpor" that conserves energy stores, as earlier hypothesized for freshwater limpets (Burky, 1969, 1971) and for hylid frogs (Packard, 1972). It may also function to lower oxygen demands at times when oxygen availability is reduced due to hypoxic conditions, and a switch to cutaneous respiration in overwintering individuals as has been hypothesized for goldfish (Roberts, 1960, 1966) and gammarids (Krog, 1954).

As *M. bidentatus*, whether cold or warm acclimated, has little or no ability to regulate $\dot{V}O_2$ in declining O_2 concentrations (Fig. 3), reverse acclimation in this species is *not* explainable as a shift to greater regulation in winter-conditioned individuals, as an adaptation of hypoxic overwintering conditions, as McMahon (1973) claimed for a freshwater pulmonate limpet, *Laevapex fuscus*. The over-

wintering environment of *M. bidentatus* cannot be characterized as one of highly fluctuating temperatures, and thus cannot correspond to Calow's (1975) hypothesis on the adaptive advantage of reverse acclimation in freshwater gastropods. The phenomenon does not appear to be associated with any seasonal changes in growth rate and food availability, as hypothesized by Parry (1978). Indeed, an excess of food was provided to both 10°C and 20°C acclimation groups throughout our experiments. Other molluscs do display seasonal patterns of acclimation, with slow growing cold-conditioned overwintering individuals having higher values of $\dot{V}O_2$ at any one temperature than do the faster growing warm-conditioned spring individuals. This clearly occurs in the freshwater clam *Pisidium walkeri* (Burky and Burky, 1976; Burky, personal communication).

M. bidentatus is apparently not as well adapted to temporarily hypoxic conditions as are several species of freshwater pulmonates and marine prosobranchs that display little or no oxygen debt after long periods of anoxia (von Brand, 1946; von Brand and Mehlmán, 1953; von Brand *et al.*, 1950; McMahon, 1973; McMahon and Russell-Hunter, 1974, 1978; deZwaan and Wijsman, 1976). Unlike these other species, *M. bidentatus* does not regulate $\dot{V}O_2$ with declining O_2 concentration (see Fig. 3). It has to repay a considerable O_2 debt, maintaining an elevated $\dot{V}O_2$ for at least 2 h after a 5 h period of anoxia (Fig. 4a). Retention of metabolic end products and subsequent oxygen debt payment had been reported as characteristic of other species of freshwater pulmonates that were more intolerant of anoxia (von Brand and Mehlmán, 1953; von Brand *et al.*, 1950). Similar oxygen debt payment has been recently reported for the intertidal prosobranchs *Littorina littorea* and *L. saxatilis* (McMahon and Russell-Hunter, 1978). Like *M. bidentatus*, these two species rarely experience hypoxic conditions in their more aerial high littoral environments, while low intertidal and subtidal gastropods (associated with aquatic environments in which hypoxic conditions are more likely to occur) generally do not display oxygen debt payment even after long periods of anoxia (McMahon and Russell-Hunter, 1974, 1978).

M. bidentatus does, however, display a short-term compensatory response to hypoxia marked by higher posthypoxic O_2 uptake rates at all O_2 tensions below air saturation, and by a distinct shift to stricter O_2 regulation of $\dot{V}O_2$ in posthypoxic individuals (Fig. 4b). Hence, posthypoxic individuals can maintain $\dot{V}O_2$ under concentrations as low as 30–60 torr (19%–38% of full air saturation with O_2) at levels equivalent to prehypoxic rates of O_2 uptake at full air saturation with O_2 ($PO_2 = 160$ torr). Such a compensatory response can allow posthypoxic individuals to maintain nearly normal levels of activity and metabolism during occasional short periods of hypoxia. Short-term hypoxic conditions can occur in both the shallow surface and subsurface waters of estuaries and salt marshes inhabited by *M. bidentatus*. Respiratory compensation for hypoxia of this type has been reported for a freshwater anyclid pulmonate, *Laevapex fuscus* (McMahon, 1972, 1973), an intertidal bivalve, *Mytilus edulis* (Bayne, 1975), several intertidal and subtidal prosobranchs (McMahon and Russell-Hunter, 1978), and most recently for a primitive estuarine pulmonate, *Amphibola crenata* (Shumway, 1981). *M. bidentatus* and *A. crenata* are amphibious air-breathers, in contrast to the more aquatic species of intertidal gastropods showing such compensatory responses to hypoxia (McMahon and Russell-Hunter, 1978). However, both of these species can commonly encounter hypoxic conditions when burrowing into the substratum for overwintering, or to avoid high tides, desiccation, or high temperatures (Hausman, 1932; Apley, 1967, 1970; Price, 1976, 1980; Watters, 1964; Shumway, 1981).

A. crenata appears to be relatively more aquatic than *M. bidentatus*; it is

submerged on every high tide. It is able to maintain equivalent levels of $\dot{V}O_2$ in both air and water and displays a greater degree of O_2 regulation and respiratory compensation to hypoxia (Shumway, 1981) than does *M. bidentatus*. However, the ability to compensate for hypoxia may be of greatest advantage to the latter species during overwintering periods, when its encounters with low ambient oxygen concentrations may be more frequent.

Freshly taken from the field population, individuals of *M. bidentatus* have a clear circadian rhythm of aerial oxygen consumption. $\dot{V}O_2$ remains above average during dark evening hours (2000–0900 h) and below average during daylight hours (0900–2000 h) with maximum values occurring near 2359 h and minimum values near 1200 h (Fig. 5). There were no discernable tidal effects on the respiratory rhythms of *M. bidentatus* (Fig. 5). Similar circadian components in respiratory rhythms and activity (with maximum values at night or early morning and minimum values during the mid or late afternoon) have been described for high salt-marsh carabs of the genus *Uca* (Brown *et al.*, 1954, 1956; Bennett *et al.*, 1957) and for the intertidal snails *Nassarius obsoletus*, *Littorina littorea*, and *Urosalpinx cinereus* (Brown *et al.*, 1958; Sandeen *et al.*, 1954). All these more aquatic species also have a persistent tidal or circatidal rhythm of activity and metabolic rate not detected in *M. bidentatus*.

In its high salt-marsh habitat, *M. bidentatus* can remain for 4–8 days without tidal submergence during each semilunar period between spring tides (Apley, 1967, 1970; Russell-Hunter *et al.*, 1972; Price, 1976, 1979, 1980). As do other extremely high littoral species, it has highly pronounced circadian (and weaker circatidal rhythms) in its activity and metabolic rates. In contrast, species from lower littoral environments, experiencing both tidal inundations and aerial exposure on a daily basis, tend to have both circadian and circatidal metabolic and activity rhythms (Palmer, 1974, 1976; Naylor, 1976; and references therein).

The pronounced circadian respiratory rhythm of *M. bidentatus* corresponds closely with reports of its field behavior. Apley (1967, 1970) states that *M. bidentatus* is inactive during periods of high mid-day ambient temperature. Cryptic behavior patterns allow *Melampus* to avoid desiccating conditions and to seek moist microhabitats. Summer-conditioned individuals of *M. bidentatus* are highly tolerant of short-term exposures to temperatures as great as 44–46°C (this study). However, *Melampus* is an extremely poor regulator of body water, losing approximately 25%–60% of total body water after 20 h exposure to relative humidities of 0%–95% (Russell-Hunter and Meadows, 1965; Price, 1976, 1980). Individuals of *M. bidentatus* undergoing desiccation have been shown to aggregate under both field and laboratory conditions, a behavior pattern which reduces the rate of water loss by about 25% (Price, 1980).

M. bidentatus is most active during the evening and early morning hours (Hausman, 1932) when ambient air temperature is at a minimum and relative humidity is at a maximum. This activity pattern corresponds directly with the circadian rhythm of $\dot{V}O_2$ recorded in this study. Also corresponding to increased activity during dark hours is the habit of carrying out 90% of egg-laying between 2359 h and 0800 h in both field populations and laboratory-held specimens (Russell-Hunter *et al.*, 1972). The reversal of metabolic rhythms in laboratory-maintained individuals (see Results and Fig. 5) may indicate that daily fluctuations of field ambient temperature and relative humidity are more important than light:dark cycles in entraining respiratory rhythms in *Melampus bidentatus*.

However, the light-dark cycle in *M. bidentatus* is important in timing the seasonal reproductive cycle with ratios greater than 14L:10D (and temperatures

greater than 15°C) required for the initiation of copulation and oviposition (Apley, 1967; Price, 1976, 1979). A strict semilunar periodicity also is associated with egg-laying. Aggregation, copulation, and oviposition occur at successive new and full moons associated with peak spring tides during the annual reproductive period, which extends from late May through early July in the Little Sippewisset marsh (Russell-Hunter *et al.*, 1972). Semilunar periodicity of reproduction is retained by specimens held in the laboratory under constant conditions (Apley, 1967, 1970; Russell-Hunter *et al.*, 1972; Price, 1976, 1979). (Seasonal and semilunar cycles of neurosecretion associated with the reproductive cycle have also been described for *M. bidentatus* [Price, 1976, 1979].)

In its natural history, ecology, and physiology, *Melampus bidentatus* is remarkably well adapted to life in the high littoral salt-marsh environment. Retaining a planktonic veliger along with an extremely high numerical fecundity (with obvious advantages to dispersal), it has evolved an elaborate semilunar pattern of reproduction and development that allows transport both of newly hatched veligers from, and of metamorphosing individuals back into, the upper reaches of the salt-marsh (Russell-Hunter *et al.*, 1972). During these events, there is a remarkable heterostrophic change in the growth patterns of the shell and mantle edge (Russell-Hunter *et al.*, 1972). They change from production of a low spired, nearly planospiral, sinistrally coiled, inflated shell (associated with the pelagic habit of the veliger) to generation of a truly turbinate, dextrally coiled shell with a narrower aperture in the metamorphosed spat. This second pattern is adapted to crawling on the marsh surface, desiccation resistance, and predator protection for the adult.

To this list of adaptations in *M. bidentatus* we can now add: (a) an extremely high thermal tolerance in both air and water as an adaptation to the temperature variation at the salt-marsh surface; (b) a high tolerance of submergence; (c) tolerance of varying salinities with little detectable effects of salinity on aquatic O₂ uptake rates; (d) a highly efficient aerial gas exchange at three to five times the rate maintained in water based upon the mantle-cavity diffusion lung and loss of the gill; (e) regulation of $\dot{V}O_2$ over the ambient temperature range allowing maintenance of a relatively efficient metabolic rates in an environment characterized by a high degree of temperature instability; (f) reverse acclimation of O₂ consumption rate when cold-conditioned as an adaptation that lowers O₂ demand and conserves energy stores in overwintering individuals (inactive-nonfeeding) entering hypoxic conditions in the salt-marsh substratum; (g) ability to compensate aquatic O₂ uptake rates to short-term exposures to hypoxia that allows near normal levels of $\dot{V}O_2$ to be maintained at O₂ tensions as low as 30–60 torr as can periodically occur in salt-marsh surface and subsurface waters; and (h) a clear circadian rhythm entrained to temperature and relative humidity variations (activity and metabolic rate are at a maximum during the dark hours of the day when desiccating conditions are minimal).

Morton (1955) describes the Ellobiidae, of which *Melampus bidentatus* is a member, as the most primitive pulmonate family, whose high littoral habitats foreshadow the purely terrestrial existence of the advanced stylommatophorans. *M. bidentatus* appears to lie very close to the terrestrial end of the gradient of evolved morphological, reproductive, and physiological characteristics associated with life in the intertidal environment. The higher pulmonates (Stylommatophora and Basommatophora) were presumably similarly preadapted for successful non-marine life (Russell-Hunter, 1979). In *M. bidentatus* the high temperature tolerance, high tolerance of desiccation, efficient aerial mode of oxygen consumption, temperature regulation of O₂ uptake, ability to enter extended overwintering periods of torpor

associated with reverse respiratory acclimation to temperature, and marked circadian periodicity of metabolism and activity, are all adaptations closely associated with the terrestrial schesis of stylommatophoran pulmonates (see Peake, 1978; Ghiretti and Ghiretti-Magaldi, 1975; Machin, 1975; Boer and Joosse, 1975; and Duncan, 1975, for discussion of such adaptations in terrestrial pulmonates).

Along with these more characteristically "terrestrial" adaptations, *M. bidentatus* retains a high tolerance of submergence (including both ability to withstand long periods of purely cutaneous aquatic gas exchange, and a high degree of salinity tolerance), relatively poor control of body water loss, a free-swimming pelagic veliger, and a semilunar reproductive cycle. These latter features are more closely associated with a littoral environment periodically and predictably inundated by high spring tides. Of these more aquatic features, retention by *M. bidentatus* of a planktonic veliger (and of the semiunar egg-laying cycle associated with the flooding of its high salt-marsh habitat by spring tides) appears to be by far the most important limiting factor that prevents this species from evolving a more terrestrial mode of life.

Of the approximately 22 genera of Ellobiids, only two, *Carychium* and *Zospeum*, have evolved a totally terrestrial mode of existence (Morton, 1955; Hubendick, 1978). *Carychium tridentatum* occurs far inland, in deciduous forests but, like *M. bidentatus*, is cryptic and limited to very humid, moist microenvironments within damp forest floor leaf litter (Boycott, 1934; Watson and Verdcourt, 1953). *Zospeum* is limited to moist cave habitats in Europe (Hubendick, 1978). The ability of *Carychium* and *Zospeum* to lay tough, leathery terrestrial eggs in which larval stages are suppressed and from which hatch miniature adults ready to take up life in a terrestrial habitat, has allowed them (and, by inference, all of the higher stylommatophoran pulmonates) to escape the intertidal habitat to which the vast majority of ellobiid genera are presently restricted, and to evolve a completely terrestrial mode of life. This implies that selection pressures characteristic of life on the high littoral salt-marsh surface must account for the evolutionary origins of most of the physiological adaptations (specifically reported here for *M. bidentatus*) that are required for a purely terrestrial habit. Subsequent invasion of moist terrestrial environments from the high littoral required only the additional evolution of a terrestrial reproductive schesis involving larger, nearly cleidoic, eggs.

LITERATURE CITED

- APLEY, M. L. 1967. Field and experimental studies on pattern and control of reproduction in *Melampus bidentatus* (Say). Ph.D. dissertation, Syracuse University. 154 pp.
- APLEY, M. L. 1970. Field studies on life history, gonadal cycle and reproductive periodicity in *Melampus bidentatus* (Pulmonata: Ellobiidae). *Malacologia* 10: 381-397.
- BARNES, H., M. BARNES, AND D. M. FINLAYSON. 1963. The seasonal changes in body weight, biochemical composition, and oxygen uptake of two common arctic cirripedes, *Balanus balanoides* and *B. balanus*. *J. Mar. Biol. Assoc. U. K.* 43: 185-211.
- BAYNE, B. L. 1975. Aspects of the physiological condition in *Mytilus edulis* L., with special reference to the effects of oxygen tension and salinity. Pp. 213-238 in H. Barnes, Ed., *Proceedings of the ninth European marine biology symposium*, Aberdeen University Press, Aberdeen.
- BENNETT, M. F., J. SHRINER, AND R. A. BROWN. 1957. Persistent tidal cycles of spontaneous motor activity in the fiddler crab, *Uca pugnax*. *Biol. Bull.* 112: 267-275.
- BERG, K. 1951. On the respiration of some molluscs from running and stagnant water. *Annee Biol.* 55: 561-563.
- BERG, K. 1952. On the oxygen consumption of Ancyliidae (Gastropoda) from an ecological point of view. *Hydrobiologia* 5: 225-267.
- BERG, K. 1953. The problem of respiratory acclimation. *Hydrobiologia* 6: 331-350.
- BERG, K., J. LUMBYE, AND K. W. OCKELMANN. 1958. Seasonal and experimental variations of the oxygen consumption of the limpet *Ancylus fluviatilis*. *J. Exp. Biol.* 35: 43-73.

- BLAZKA, P. 1954. Temperaturadaptation des gesamtmetabolismus bei der weinbergschnecke *Helix pomatia* L. *Zool. Jahr. Abt. Allg. Zool. Physiol.* **65**: 130-138.
- BOER, H. H., AND J. JOOSSE. 1975. Endocrinology. Pp. 245-307 in V. Fretter and J. Peake, Eds., *Pulmonates, Vol. 1, Functional anatomy and physiology*. Academic Press, London.
- BOYCOTT, A. E. 1934. The habitats of land mollusca in Britain. *J. Ecol.* **22**: 1-388.
- VON BRAND, T. 1946. Anaerobiosis in invertebrates. *Biodynamica Monographs* **4**: 1-328.
- VON BRAND, T., H. D. BAERNSTEIN, AND B. MEHLMAN. 1950. Studies on the anaerobic metabolism and anaerobic carbohydrate consumption of some freshwater snails. *Biol. Bull.* **98**: 266-276.
- VON BRAND, T., AND B. MEHLMAN. 1953. Relations between pre- and post anaerobic oxygen consumption and oxygen tension in some freshwater snails. *Biol. Bull.* **104**: 301-312.
- BROEKHUYSEN, G. J. 1940. A preliminary investigation of the importance of desiccation, temperature, and salinity as factors controlling the vertical distribution of certain intertidal marine gastropods in False Bay, South Africa. *Trans. R. Soc. S. Afr.* **28**: 255-291.
- BROWN, F. A., JR., M. F. BENNETT, AND H. M. WEBB. 1954. Persistent daily and tidal rhythms of O₂-consumption in fiddler crabs. *J. Cell. Comp. Physiol.* **44**: 477-506.
- BROWN, F. A., JR., W. J. BRETT, AND H. M. WEBB. 1958. The rhythmic nature of metabolism in *Ilyanassa* in constant conditions. *Biol. Bull.* **115**: 345.
- BROWN, F. A., JR., R. A. BROWN, H. M. WEBB, M. BENNETT, AND J. SHRINER. 1956. A persistent tidal rhythm of locomotor activity in *Uca pugnax*. *Anat. Rec.* **125**: 613-614.
- BURKY, A. J. 1969. Biomass turnover, energy balance, and interpopulation variation in the stream limpet, *Ferrissia rivularis* (Say), with special reference to respiration, growth, and fecundity. Ph.D. dissertation, Syracuse University. 104 pp.
- BURKY, A. J. 1970. Reverse acclimation at low temperature in the stream limpet, *Ferrissia rivularis* (Say). *Acta Cient. Venez.* **21**: 28.
- BURKY, A. J. 1971. Biomass turnover, respiration, and interpopulation variation in the stream limpet, *Ferrissia rivularis* (Say). *Ecol. Monogr.* **41**: 235-251.
- BURKY, A. J. 1977. Respiration chambers for measuring oxygen consumption of small aquatic molluscs with Clark-type polarographic electrodes. *Malacol. Rev.* **10**: 71-72.
- BURKY, A. J., AND K. A. BURKY. 1976. Seasonal respiratory variation and acclimation in the pea clam, *Pisidium walkeri* Sterki. *Comp. Biochem. Physiol.* **55A**: 109-114.
- CALOW, P. 1975. The respiratory strategies of two species of freshwater gastropods (*Ancylus fluviatilis* Müll and *Planorbis contortus* Linn) in relation to temperature, oxygen concentration, body size, and season. *Physiol. Zool.* **48**: 114-129.
- CAVENAUGH, G. M. 1956. *Formulae and Methods VI of the Marine Biological Laboratory Chemical Room*. Marine Biological Laboratory, Woods Hole, Massachusetts. Pp. 62-69.
- DAVIES, P. S. 1965. Environmental acclimation in the limpet, *Patella vulgata* L. *Nature* **205**: 924.
- DUNCAN, A. 1966. The oxygen consumption of *Potamopyrgus jenkinsi* (Smith) (Prosobranchiata) in different temperatures and salinities. *Verh. int. Ver. Limnol.* **16**: 1739-1751.
- DUNCAN, A., AND R. Z. KLEKOWSKI. 1967. The influence of salinity on the survival, respiratory rate and heart beat of young *Potamopyrgus jenkinsi* (Smith) Prosobranchiata. *Comp. Biochem. Physiol.* **22**: 495-505.
- DUNCAN, C. J. 1975. Reproduction. Pp. 309-365 in V. Fretter and J. Peake, Eds., *Pulmonates, Vol. 1, Functional anatomy and physiology*. Academic Press, London.
- EVANS, R. G. 1948. The lethal temperatures of some common British littoral molluscs. *J. Anim. Ecol.* **17**: 165-173.
- FARNIE, W. C. 1919. The structure of *Amphibola crenata* (Martyn). *Trans. Proc. N. Z. Inst.* **51**: 69-85.
- FISCHER, P.-H., M. DUVAL, AND A. RAFFY. 1933. Études sur les échanges respiratoires des littorines. *Arch. Zool. Exp. Gen.* **74**: 627-634.
- FRAENKEL, G. 1961. Resistance to high temperatures in a Mediterranean snail, *Littorina neritoides*. *Ecology* **42**: 604-606.
- FRAENKEL, G. 1966. The heat resistance of intertidal snails at Shirahama, Wakayama-ken, Japan. *Publ. Seto. Mar. Biol. Lab.* **14**: 185-195.
- FRAENKEL, G. 1968. The heat resistance of intertidal snails at Bimini, Bahamas; Ocean Springs, Mississippi; and Woods Hole, Massachusetts. *Physiol. Zool.* **41**: 1-13.
- GHIRETTI, F., AND A. GHIRETTI-MAGALDI. 1975. Respiration. Pp. 33-52 in V. Fretter and J. Peake, Eds., *Pulmonates, Vol. 1, Functional Anatomy and Physiology*. Academic Press, London.
- GODDARD, C. K., AND A. W. MARTIN. 1966. Carbohydrate metabolism. Pp. 275-308 in K. M. Wilbur and C. M. Yonge, Eds., *Physiology of Mollusca, Vol. II*. Academic Press, London.
- GOWANLOCH, J. N., AND F. R. HAYES. 1926. Contributions to the study of marine gastropods. I. The physical factors, behavior, and intertidal life of *Littorina*. *Contrib. Can. Biol. Fish.* **3**: 133-165.

- HILBISH, T. J. 1981. Latitudinal variation in freezing tolerance of *Melampus bidentatus* (Say) (Gastropoda: Pulmonata). *J. Exp. Mar. Biol. Ecol.* **52**: 283–297.
- HAUSMAN, S. A. 1932. A contribution to the ecology of the salt marsh snail, *Melampus bidentatus* Say. *Am. Nat.* **66**: 541–545.
- HUBENDICK, B. 1978. Systematics and comparative morphology of the Basommatophora. Pp. 1–47, in V. Fretter and J. Peake, Eds., *Pulmonates, Vol. 2A, Systematics, evolution and ecology*. Academic Press, London.
- KINNÉ, O. 1964. The effects of temperature and salinity on marine and brackish water animals II. Salinity and temperature salinity combinations. *Oceanogr. Mar. Biol. Ann. Rev.* **2**: 281–339.
- KINNÉ, O. 1971. Salinity—invertebrates. Pp. 821–995, in O. Kinné, Ed., *Marine ecology, Vol. I*. Wiley-Interscience, New York.
- KROG, H. 1954. Temperature sensitivity of freshwater gammarids. *Biol. Bull.* **107**: 397–410.
- LEWIS, J. B. 1963. Environmental and tissue temperatures of some tropical intertidal marine animals. *Biol. Bull.* **124**: 277–284.
- LOCKWOOD, A. P. M. 1976. Physiological adaptation to life in estuaries. Pp. 315–392 in R. C. Newell, Ed., *Adaptation to Environment: Essays on the Physiology of Marine Animals*. Butterworth and Co., Ltd., London.
- LUMBYE, J. 1958. The oxygen consumption of *Theodoxus fluviatilis* (L.) and *Potamopyrgus jenkinsi* (Smith) in brackish and fresh water. *Hydrobiologia* **10**: 245–262.
- LUMBYE, J., AND L. E. LUMBYE. 1965. The oxygen consumption of *Potamopyrgus jenkinsi* (Smith). *Hydrobiologia* **25**: 489–500.
- MACHIN, J. 1975. Water relationships. Pp. 105–163, in V. Fretter and J. Peake, Eds., *Pulmonates, Vol. 1, Functional Anatomy and Physiology*. Academic Press, London.
- MANGUM, C. P., AND W. VAN WINKLE. 1973. Responses of aquatic invertebrates to declining oxygen conditions. *Am. Zool.* **13**: 529–541.
- MCMAHON, R. F. 1972. Interpopulation variation and respiratory acclimation in the bioenergetics of *Laevapex fuscus*. Ph.D. dissertation, Syracuse University. 164 pp.
- MCMAHON, R. F. 1973. Respiratory variation and acclimation in the freshwater limpet, *Laevapex fuscus*. *Biol. Bull.* **145**: 492–508.
- MCMAHON, R. F. 1975. Growth, reproduction and bioenergetic variation in three natural populations of a freshwater limpet *Laevapex fuscus* (C. B. Adams). *Proc. Malacol. Soc. Lond.* **41**: 331–351.
- MCMAHON, R. F. 1976. Effluent-induced interpopulation variation in the thermal tolerance of *Physa virgata* Gould. *Comp. Biochem. Physiol.* **55A**: 23–28.
- MCMAHON, R. F., AND B. S. PAYNE. 1980. Variation of thermal tolerance limits in populations of *Physa virgata* Gould (Mollusca: Pulmonata). *Am. Midl. Nat.* **103**: 218–230.
- MCMAHON, R. F., AND W. D. RUSSELL-HUNTER. 1974. Responses to low oxygen stress in relation to the ecology of littoral and sub-littoral snails. *Biol. Bull.* **147**: 490.
- MCMAHON, R. F., AND W. D. RUSSELL-HUNTER. 1977. Temperature relations of aerial and aquatic respiration in six littoral snails in relation to their vertical zonation. *Biol. Bull.* **152**: 182–198.
- MCMAHON, R. F., AND W. D. RUSSELL-HUNTER. 1978. Respiratory responses to low oxygen stress in marine littoral and sublittoral snails. *Physiol. Zool.* **51**: 408–424.
- MORTON, J. E. 1955. The evolution of the Ellobiidae with a discussion of the origin of the pulmonata. *Proc. Zool. Soc. Lond.* **125**: 127–168.
- NAYLOR, E. 1976. Rhythmic behavior and reproduction in marine animals. Pp. 393–429 in R. C. Newell, Ed., *Adaptation to environment: Essays on the physiology of marine animals*. Butterworth and Co., Ltd., London.
- NEWELL, R. C. 1973. Factors affecting the respiration of intertidal invertebrates. *Am. Zool.* **13**: 513–528.
- NEWELL, R. C. 1976. Adaptations to intertidal life. Pp. 1–82 in R. C. Newell, Ed., *Adaptation to environment: Essays on the physiology of marine animals*. Butterworth and Co., Ltd., London.
- NEWELL, R. C. 1979. *Biology of intertidal animals*, Third edition. Marine Ecological Survey Ltd., Faversham, Kent, U. K., 781 pp.
- NEWELL, R. C., AND V. I. PYE. 1970a. Seasonal changes in the effect of temperature on the oxygen consumption of the winkle *Littorina littorea* (L.) and the mussel *Mytilus edulis* L. *Comp. Biochem. Physiol.* **34**: 367–383.
- NEWELL, R. C. AND V. I. PYE. 1970b. The influence of thermal acclimation on the relation between oxygen consumption and temperature in *Littorina littorea* (L.) and *Mytilus edulis* L. *Comp. Biochem. Physiol.* **34**: 385–397.
- NEWELL, R. C., AND V. I. PYE. 1971a. Quantitative aspects of the relationship between metabolism and temperature in the winkle *Littorina littorea* (L.). *Comp. Biochem. Physiol.* **38B**: 635–650.

- NEWELL, R. C., AND V. I. PYE. 1971b. Temperature induced variations in the respiration of mitochondria from the winkle *Littorina littorea* (L.). *Comp. Biochem. Physiol.* **40B**: 249-261.
- NEWELL, R. C., AND A. ROY. 1973. A statistical model relating the oxygen consumption of a mollusc (*Littorina littorea*) to activity, body size, and environmental conditions. *Physiol. Zool.* **46**: 253-275.
- PACKARD, G. C. 1972. Inverse compensation for temperature in oxygen consumption of the hyliid frog *Pseudacris triseriata*. *Physiol. Zool.* **45**: 270-275.
- PALMER, J. D. 1974. *Biological clocks in marine organisms: The control of physiological and behavioral tidal rhythms*. John Wiley and Sons, New York. 173 pp.
- PALMER, J. D. 1976. *An introduction to biological rhythms*. Academic Press, London. 375 pp.
- PARRY, G. D. 1978. Effects of growth and temperature acclimation on metabolic rate in the limpet, *Cellana tramoserica* (Gastropoda: Patellidae). *J. Anim. Ecol.* **47**: 351-368.
- PEAKE, J. 1978. Distribution and ecology of the stylommatophora. Pp. 429-526 in V. Fretter and J. PEAKE, Eds., *Pulmonates, Vol. 2A. Systematics, evolution and ecology*. Academic Press, London.
- PRICE, C. H. 1976. Behavior, physiological ecology, and neurosecretion in the reproduction and water relations of *Melampus bidentatus* Say (Mollusca: Pulmonata). Ph.D. dissertation, Syracuse University, 172 pp.
- PRICE, C. H. 1979. Physical factors and neurosecretion in the control of reproduction in *Melampus* (Mollusca: Pulmonata). *J. Exp. Zool.* **207**: 269-282.
- PRICE, C. H. 1980. Water relations and physiological ecology of the salt marsh snail, *Melampus bidentatus* Say. *J. Exp. Mar. Biol. Ecol.* **45**: 51-67.
- PROSSER, C. L. 1973. Temperature. Pp. 362-428 in C. L. Prosser, Ed., *Comparative animal physiology*, Third ed. W. B. Saunders Company, Philadelphia.
- RAFFY, A. 1933. Recherches sur le métabolisme respiratoire des poikilothermes aquatiques. *Ann. Inst. Océanogr. Monaco* **13**: 263-396.
- ROBERTS, J. L. 1960. The influence of photoperiod on thermal acclimation. *Zool. Anz.* **24**: 73-78.
- ROBERTS, J. L. 1966. Systematic versus cellular acclimation to temperature by poikilotherms. *Helgolander Wiss. Meeresunters.* **14**: 451-465.
- RUSSELL-HUNTER, W. D. 1979. *A life of invertebrates*. Macmillan Publishing Company, New York. 650 pp.
- RUSSELL-HUNTER, W. D., M. L. APLEY, AND R. D. HUNTER. 1972. Early life-history of *Melampus* and the significance of semilunar synchrony. *Biol. Bull.* **143**: 623-656.
- RUSSELL-HUNTER, W. D., AND A. G. EVERSOLE. 1976. Evidence for tissue degrowth in starved freshwater pulmonate snails (*Helisoma trivolvis*) from tissue, carbon and nitrogen analysis. *Comp. Biochem. Physiol.* **54A**: 447-453.
- RUSSELL-HUNTER, W. D., AND R. L. MEADOWS. 1965. Aspects of water physiology in the salt-marsh pulmonate snail, *Melampus bidentatus*. *Biol. Bull.* **129**: 409.
- SANDEEN, M. I., C. G. STEPHENS, AND F. A. BROWN, JR. 1954. Persistent daily and tidal rhythms of oxygen consumption in two species of marine snails. *Physiol. Zool.* **27**: 350-356.
- SANDISON, E. E. 1967. Respiratory response to temperature and temperature tolerance of some intertidal gastropods. *J. Exp. Mar. Biol. Ecol.* **1**: 271-281.
- SCHLIEPER, C. 1971. Physiology of brackish water. Part II. Pp. 211-250 in A. Remane, and C. Schlieper (Authors), *Biology of brackish water*. John Wiley and Sons, Inc., New York.
- SHIRLEY, T. C., G. Y. DENOUE, AND W. B. STICKLE. 1978. Seasonal respiration in the marsh periwinkle, *Littorina irrora*. *Biol. Bull.* **154**: 322-334.
- SHUMWAY, S. E. 1981. Factors effecting oxygen consumption in the marine pulmonate *Amphibola crenata* (Gmelin, 1791). *Biol. Bull.* **159**: 332-347.
- TOULMOND, M. A. 1967a. Étude de la consommation d'oxygène en fonction du poids, dans l'air et dans l'eau, chez quatre espèces du genre *Littorina* (Gastropoda, Prosobranchiata). *C. R. Hebd. Seances Acad. Sci. Paris* **264**: 636-638.
- TOULMOND, A. 1967b. Consommation d'oxygène, dans l'air et dans l'eau, chez quatre gastéropodes du genre *Littorina*. *J. Physiol. (Paris)* **59**: 303-304.
- WATSON, H., AND B. VERDCOURT. 1953. The two British species of *Carychium*. *J. Conchol.* **23**: 306-324.
- WATTERS, P. A. 1964. Distribution of *Amphibola crenata* (Pulmonata) in the Dunedin area, with notes on the probable origin of the species. *Trans. R. Soc. New Zealand*, **4**: 117-134.
- DE ZWAAN, A., AND T. C. M. WIJSMAN. 1976. Anaerobic metabolism in Bivalvia (Mollusca). Characteristics of anaerobic metabolism. *Comp. Biochem. Physiol.* **54B**: 313-324.